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98-2-255

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Genotypic variation in carbon isotope discrimination and gas exchange of mature ponderosa pine trees at two locations in the Great Plains

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Final Report for 28-C2-599, PI = Thomas C. Hennessey
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Abstract: Genotypic variation in carbon isotope discrimination (Δ), photosynthetic gas exchange, water relations, and needle morphology traits were analyzed in four ponderosa pine (*Pinus ponderosa* Doug. ex Laws.) seed sources growing in two 26-year-old provenance plantings, near Plattsmouth, NE and Norman, OK. The seed sources studied were from northern South Dakota, southern New Mexico, eastern Wyoming and north central Nebraska. These seed sources had shown a genotype x environment (*G x E*) interaction for growth and needle morphology in previous studies. Net photosynthesis (*A*), needle conductance to water vapor (g_{wv}), transpiration (*E*), and xylem pressure potential (Ψ_x) were measured during the growing season of 1994. Specific leaf area (*SLA*), stomatal density, and Δ were analyzed in needles grown from 1991 to 1994. Neither genotypic variation nor *G x E* interaction in *A*, g_{wv}, or *E* were detected in the ponderosa pine sources studied. Intrinsic water use efficiency (A/g_{wv}) was highest in the most southern source, and predawn Ψ_x was significantly higher in this same source. However, a *G x E* interaction was indicated for predawn Ψ_x.

There was a positive correlation between Δ and g_{wv}, and a negative correlation between Δ and A/g_{wv}. Thus, we speculate that the variation in Δ in ponderosa pine is more related to g_{wv} than to *A*. Although *SLA* and stomatal density were significantly different among seed sources and among years, Δ was only weakly correlated with stomatal

density. Thus, needle morphology does not explain variation in Δ in ponderosa pine trees. There was a significant genotypic variation and seed source x location interaction in Δ, indicating a *G x E* interaction in this trait. Trees from New Mexico showed low Δ and trees from South Dakota exhibited high Δ at both locations. In contrast, trees from Nebraska exhibited high Δ at Plattsmouth, but low Δ at Norman; and trees from Wyoming showed low Δ at Plattsmouth and high Δ at Norman. Although there was a strong relationship between the variation in Δ and the variation in rainfall-potential evapotranspiration (ETp) deficit among years and between locations, the rank order in Δ of the seed sources studied was essentially the same through the years at both locations. These results indicate a lack of *G x E* interaction in Δ due to precipitation. Therefore, we speculate that the *G x E* interaction detected based on the seed source x location interaction, may be more related to latitude, photoperiod, temperature, or edaphic characteristics than to precipitation.

Introduction

Several recent studies have demonstrated significant genotypic variation in carbon isotope discrimination (Δ) in crops and forest trees (Farquhar *et al.* 1989). The isotopic ratio of ^{13}C to ^{12}C in C_3 plants varies mainly due to discrimination in diffusion and enzymatic processes. Diffusivity of $^{13}CO_2$ across the stomatal pathway is lower than that of $^{12}CO_2$ by a factor of 4.4‰. Additionally, there is a differentiation in reaction caused by the preference of ribulose bisphosphate carboxylase (*Rubisco*) for $^{12}CO_2$ over $^{13}CO_2$ (by a factor of ~27‰). In both cases, the processes discriminate against the heaviest isotope

^{13}C. Furthermore, the amount of discrimination against ^{13}C in photosynthesis has been shown to be highly negatively correlated with water use efficiency (Condon *et al.* 1990, Condon *et al.* 1992, Hubick *et al.* 1986, and Wright *et al.* 1994).

The correlation between water use efficiency and Δ has been extensively studied in several crops including common bean (*Phaseolus vulgaris* L.) (Ehleringer 1990, Ehleringer *et al.* 1991), wheat (*Triticum aestivum* L.) (Farquhar and Richards 1984 and Condon *et al.* 1990), peanut (*Arachis hypogea* L.) (Hubick *et al.* 1986 and Wright *et al.* 1994), barley (*Hordeum vulgare* L.) (Febrero *et al.* 1994), and cowpea (*Vigna unguiculata* [L.] Walp.) (Ismail *et al.* 1994). These studies suggest that genetic variation in Δ may be sufficient to be useful as a selection criterion for improved water use efficiency.

Analysis of Δ for improving water use efficiency in forest trees has been analyzed only in a few species including Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Zhang *et al.* 1993), black spruce (*Picea mariana* [Mill.] B.S.P.) (Johnsen *et al.* 1993), boxelder (*Acer negundo* L.) (Dawson and Ehleringer 1993), and western larch (*Larix occidentalis* Nutt.) (Zhang and Marshall 1994). In general, these studies demonstrated genetic variation in Δ, suggesting that it may be possible to use Δ as a selection criterion to improve water use efficiency and yield. However, there are no reported studies on the correlation between water use efficiency and Δ in mature ponderosa pine (*Pinus ponderosa* Doug. ex Laws.) trees.

Genetic variation in the physiological mechanisms associated with drought tolerance has been studied in several forest tree species, including loblolly pine (*Pinus taeda* L.) (van Buijtenen *et al.* 1976, Bongarten and Teskey 1986, Bongarten and Boltz 1986, Teskey *et al.* 1987, Seiler and Johnson 1988), lodgepole pine (*Pinus contorta* Dougl. ex Loud.) (L'Hirondelle 1994), black spruce (Bernier *et al.* 1994), and ponderosa pine (Monson and Grant 1989, Cregg 1993*a*, 1994). These studies indicated significant, and potentially exploitable, intraspecific variation in a number of morphophysiological traits related to drought tolerance including rate of gas exchange, osmotic adjustment, and stomatal density. Further, we currently have a limited understanding of the genotype x environment (*G x E*) interaction in morphophysiological traits related to drought tolerance. The importance of determining if forest trees exhibit *G x E* interaction resides in that if the relative performance of genotypes is independent of the environment, the sources may be screened and selected with little regard to the location in which they will be outplanted. If, however, significant *G x E* interactions are indicated, then genotypic screening and selection criterion must be designed accordingly. In this study our objectives were to: 1) determine if there is a correlation between intrinsic water use efficiency (A/g_{wv}) and Δ in ponderosa pine; and 2) determine if there is *G x E* interaction in Δ and gas exchange in four ponderosa pine seed sources.

Materials and methods

Study locations

The study was conducted during the 1994 growing season in two of the ponderosa pine provenance trails established by the USDA Forest Service in the Great Plains in 1968 (Read 1980). Site 1 was at Horning State Farm near Plattsmouth, Nebraska, and Site 2 near Norman, Oklahoma. The plantings were established using seedlings grown in nurseries from open-pollinated seed collected in natural stands of ponderosa pine (Read 1980). Seed from each source was bulked before sowing in the nursery.

Site 1-Plattsmouth, Nebraska

This planting is located at a latitude of 41° 00' N, longitude 95° 54' W and an elevation of 335 m. The planting was established as a randomized complete block design with six blocks of 50 sources. The trees were planted at a spacing of 2.5 x 3.0 m in linear plots of 10 trees (Read 1983). The plantation is located on a west aspect with a slope of approximately 5%. The soils are classified as Marshal silty clay loam (fine-silty, mixed, mesic Typic Hapludoll) (Borchers and Witte 1984). Monthly mean temperature, precipitation, and potential evapotranspiration (ETp) are given in Fig. 1.

Site 2-Norman, Oklahoma

This planting is located at a latitude of 35° 06' N, longitude 97° 30' W, and an elevation of 335 m. The planting was installed as a randomized complete block design with 15 replications of 40 sources. The trees were planted at a spacing of 4 x 4 m in linear plots of 4 trees (Read 1983). The plantation is located on a flat area. The soils are classified as Minco silt loam (coarse-silty, mixed, thermic Udic Haplustolls) (Moebius and Sparwasser 1979). Monthly mean temperature, precipitation and ETp are given in Fig. 1.

Plant materials

We used 26-year-old trees from four ponderosa pine seed sources. The seed sources chosen for the study were: from northwestern South Dakota (704), southern New Mexico (767), eastern Wyoming (832), and north central Nebraska (856). Climatic data for the seed sources is presented in Table 1. These sources were chosen for this research because of a previous study conducted at age 15 by Van Haverbeke (1986) showed a $G \times E$ interaction in tree height between Plattsmouth and Norman locations. Additionally, in a study at age 25, Cregg (1993*b*) found a $G \times E$ interaction in needle morphology (stomates per needle and needle length) between Plattsmouth and Norman plantation locations.

At the Plattsmouth planting, we selected twelve trees from each of the seed sources, except for 767 which was represented by 11 trees. The experimental design used for this study in this planting was a randomized complete block design. Trees were grouped in three blocks (replications) of 16 trees, each containing 4 trees from each seed source, except for block 3 which included only 15 trees. Slope of the site was used as a criterion for defining each block. Additionally, trees selected within each block were located in adjacent plots to minimize the distance between study trees and time between measurements within blocks. We selected trees from each source that were free of insects, disease or other obvious defects.

At the Norman planting, six trees from each of the seed sources were selected. Each tree represented a replication in the study. The experimental design used in this

planting was a completely randomized design. The trees were selected at random from those in the source which were free of insects, disease, or other obvious defects.

Measurements

Water relations and gas exchange

At the Plattsmouth plantation xylem pressure potential (Ψ_x) was determined before dawn and at the time of each gas exchange measurement (midday Ψ_x) on one fascicle from each tree, using the pressure chamber technique (Ritchie and Hinckley 1975). Net photosynthesis (A), needle conductance to water vapor (g_{wv}), and transpiration (E) were measured with an open gas analyzer system (model LCA-4, Analytical Development Company, Hoddsen, England). Intrinsic water use efficiency was calculated by dividing A by g_{wv}. Gas exchange was measured on six dates during 1994 (March, May, June, July, September, and October). Gas exchange was measured twice in each tree on each date (once in the morning and once in the afternoon), measuring trees within each block through one day (3 days for each set of measurements). These measurements were collected on six 1-year-old needles (two or three fascicles) on three exposed branches of each tree, except for October when they were collected using current year needles. All gas exchange measurements were completed on clear days, with a mean photosynthetic photon flux density (*PPFD*) above 1200 μmol m^{-2} s^{-1} (mean=1550, SE=7).

A field weather station equipped with a data logger recorded precipitation, temperature, relative humidity, and *PPFD* at the site during the study. We measured volumetric soil moisture at approximately 1 m from each tree at depths of 30 and 60 cm

by time domain reflectometry (Trase Moisture Measuring System) (Topp and Davis 1985) on the same dates when gas exchange and plant water relations were measured.

At the Norman plantation Ψ_x and gas exchange were measured in a similar manner under a current cooperative agreement with Oklahoma State University. However, in this plantation gas exchange was measured using a gas analyzer system running as a closed-type (model CI-301, CID, Inc., Vancouver, Washington). A previous study conducted by Cregg and Hennessey (1995) indicated that it is possible to make comparisons of relative values or rank orders of gas exchange rates measured with the LCA-4 and CI-301 units. At Norman, gas exchange measurements were collected on three dates during the growing season of 1994 (May, June, and July) on days with a mean daily *PPFD* of 1670 (SE=36), 773 (SE=34) and 742 (SE=62) μmol m^{-2} s^{-1} respectively. Gas exchange was measured once on each tree on a given measurement date. In this plantation soil moisture was determined gravimetrically in July. Soil samples were collected approximately 1 m from each study tree at two depths, 23 and 46 cm.

Carbon isotope discrimination

Needle samples were collected at both plantations in June, 1994 from three exposed branches of each of the trees used for gas exchange measurements . The branches sampled were adjacent to those used for gas exchange measurements. Needles collected at this time were grown during 1991, 1992, and 1993, except in Norman where it was not possible to find living needles from 1991. In October 1994, current year needles were collected from the same branches used for the first collection.

Needles from each tree were oven-dried at 70°C for 48 h and finely ground. The relative abundance of ^{13}C and ^{12}C was determined with an isotope ratioing mass spectrometer at the Laboratory for Isotope Biodynamics, Department of Biochemistry, University of Nebraska-Lincoln. Stable carbon isotope ratio ($\delta^{13}C$) was expressed as the $^{13}C/^{12}C$ ratio relative to Pee Dee Belemnite (limestone) (Craig 1957). The resulting $\delta^{13}C$ values were used to estimate isotopic discrimination as

$\Delta = (\delta_a - \delta_p)/(1 + \delta_p)$, where δ_p is the isotopic composition of the plant material and δ_a is that of the air (assumed to be -8‰) (Farquhar *et al.* 1989).

Needle morphology

To analyze needle morphology parameters needles were collected at the same time and in the same way to those collected for carbon isotope discrimination. We measured specific leaf area (*SLA*) and stomatal density of needles grown during the growing seasons of 1991, 1992, 1993, and 1994, except for Norman where all needles from 1991 had senesced.

Projected surface area of a sample of 10 fascicles from each tree was determined with an area meter (Model LI-3100, Li-Cor, Lincoln, NE). Projected surface area was used to calculate total surface area (*TSA*) as described by Cregg (1993*a*). The number of two and three needle fascicles in the sample and the cumulative needle length were recorded. Subsequently, the needles were dried and weighed. Specific leaf area was calculated as *SLA=TSA*/dry weight (g).

Stomatal density was determined by examining one needle from each of five fascicles under a dissecting microscope. Number of stomatal rows around the needle and number of stomates within rows were counted on a 2.6-mm length segment near the middle of each needle. Number of rows of stomates was multiplied by the number of stomates per row to calculate the number of stomates per mm. Stomates per needle were estimated by multiplying stomates per mm by the needle length. Density was calculated by dividing the number of stomates per needle by total needle surface area.

Statistical analysis

Source and location effects on gas exchange and water relations were determined by a repeated measures analysis of variance (Moser and Saxton 1990) of the data collected in May, June, and July. Variation in stomatal density, *SLA*, and Δ were analyzed by analysis of variance. Where significant differences were indicated, differences in seasonal means of gas exchange traits, water relation parameters and mean *SLA*, stomatal density, and Δ were separated by Tukey's studentized range test applied at α=0.05.

The relationship between Δ and gas exchange variables was analyzed by using data of Δ in needles grown during 1994 and the mean gas exchange measured in 1994 at both locations. Simple linear regression was used to detect relationships between variables. Seed source differences in the relation of Δ to gas exchange and morphology variables were evaluated by analysis of covariance.

Results

Precipitation and potential evapotranspiration

Overall, the rainfall-ETp deficit during the growing season (May to October) from 1991 to 1994 was higher at Norman than at Plattsmouth, except in 1992 when rainfall-ETp deficit was higher at Plattsmouth than at Norman (Fig. 2). At Plattsmouth, the rainfall-ETp deficit was higher in 1991 and 1992 than in 1993 and 1994; and during 1993 ETp was lower than precipitation. At Norman, the rainfall-ETp deficit was higher in 1993 and 1994 than in 1992, a year when the deficit was close to zero.

Water relations

At Plattsmouth, soil moisture was the same for the four seed sources, and it was higher in March and June than in the other months. At Norman, soil moisture was measured only in July, and there was no difference among seed sources. At Plattsmouth, seasonal variation in predawn Ψ_x was the same for the four seed sources, except in June and July (Fig. 3). In June, trees from seed source 767 showed significantly higher predawn Ψ_x than trees from sources 704 and 856, whereas in July, trees from source 767 had significantly lower predawn Ψ_x than the rest of the trees. At this planting, midday Ψ_x was higher for trees from source 767 than for trees from source 856 on every measurement date, whereas trees from sources 704 and 832 exhibited diverse behavior during the season (Fig. 3). At Norman, there were no differences in predawn Ψ_x and midday Ψ_x among seed sources through the season.

Averaged over time, predawn Ψ_x was significantly higher in trees grown at Norman than in trees grown at Plattsmouth, and the location x seed source interaction was also significant (Fig. 4). At Plattsmouth, trees from seed source 856 showed lower

predawn Ψ_x than trees from seed source 767; and at Norman, the difference in predawn Ψ_x among seed sources was not significant. Midday Ψ_x was higher at Norman than at Plattsmouth, but there was no location x seed source interaction.

Gas exchange

Analyzing gas exchange traits by location, it was found that at Plattsmouth, there was a highly significant date x seed source effect on A. This interaction was due to a difference among sources in June, when trees from source 832 exhibited significantly lower A than trees from sources 767 and 856 (Fig. 5). Overall, A was the highest for every seed source in October, possible because on this date gas exchange was measured on current year needles. Seasonal variation in g_{wv} and E was the same for the four seed sources (Fig. 5). As for A, June was the only date when there was a significant difference among seed sources. On this date, trees from source 832 had higher g_{wv} than trees from source 767, and higher E than trees from sources 767 and 856. Needle conductance to water vapor for every seed source was higher in September and October than on the other measurement dates.

Intrinsic water use efficiency at Plattsmouth was lower in September than on the rest of the measurement dates (Fig. 5) probably due to the high g_{wv} on that date. There was a significant difference in A/g_{wv} among seed sources on three of the measurement dates. In June, trees from source 767 had higher A/g_{wv} than trees from sources 704 and 832. However, in July, A/g_{wv} was the same for trees from sources 704, 832, and 767; and

trees from source 856 had lower A/g_{wv} than trees from sources 704 and 832. Finally, in September, trees from source 767 exhibited higher A/g_{wv} than trees from source 856.

At the Norman planting, there was a significant date x seed source interaction in A, g_{wv}, and E (Fig. 5). For A, this interaction was due to a difference among seed sources in July, when trees from seed source 832 had lower A than trees from sources 704 and 767. Needle conductance to water vapor differences among seed sources were diverse on each date. In May, trees from source 832 had higher g_{wv} than trees from sources 856 and 767. In June, g_{wv} was the highest for trees from source 704, and in July, trees from source 856 had lower g_{wv} than trees from source 704. Transpiration was the lowest for trees from source 832 in June; and in July, trees from source 704 had lower E than trees from sources 832 and 856. Finally, in this planting, A/g_{wv} was the same for the four seed sources during the season.

Analyzing the gas exchange data from May, June, and July at both locations, it was found that, averaged over time, there were no differences in A, g_{wv}, and E among seed sources (Table 2). However, A/g_{wv} was significantly higher for trees from seed source 767 than for trees from the other sources (Fig. 6). The location x seed source interaction in A, E, g_{wv}, and A/g_{wv} was not significant, but there was a significant difference between locations, and significant date x location and date x seed source interactions in these traits (Table 2). Net photosynthesis, E, and g_{wv} were higher at Plattsmouth than at Norman, and A/g_{wv} was higher at Norman than at Plattsmouth (Fig. 6).

Needle morphology

The analysis of needle morphology indicated a significant difference in *SLA* and stomatal density between locations (Table 3). Overall, *SLA* was greater in trees grown at Plattsmouth than in trees grown at Norman, and the opposite was true for stomatal density. There was no location x seed source interaction in *SLA*, but in stomatal density this interaction was significant. Trees from seed source 856 exhibited lower *SLA* than trees from seed sources 832 and 767 at Plattsmouth, but within Norman there was no difference among seed sources. At Plattsmouth, stomatal density was greater for trees from seed source 856 than for trees from the other sources, and at Norman, trees from source 767 had greater stomatal density than trees from source 832. We also detected significant difference in *SLA* and stomatal density among years. At Plattsmouth, *SLA* and stomatal density were the highest in 1994, but at Norman the difference in stomatal density among years was not significant. The year x seed source interaction in *SLA* and stomatal density was not significant.

Carbon isotope discrimination

There was no difference in Δ between locations, but seed source, location x seed source interaction, year, and location x year interaction were significant (Table 4). Seed source x year and location x seed source x year interactions were not significant.

The location x seed source interaction in Δ was due to trees from seed sources 832 and 856 (Fig. 7). At Plattsmouth, trees from seed source 767 exhibited lower Δ than trees from sources 856 and 704; and at Norman, trees from seed sources 767 and 856 exhibited lower Δ than the other two sources. Additionally, the magnitude of seed source

differences in Δ was larger at Norman than at Plattsmouth. The location x year interaction was due to a higher Δ in 1993 and 1994 than in 1992 at Plattsmouth; and at Norman, Δ was higher in 1992 and 1993 than in 1994. The year x seed source interaction within each location was not significant.

The analysis of the relationship between Δ and the physiological and morphological variables evaluated (Fig. 8) indicated a lack of correlation between Δ and A at both locations (Plattsmouth, r=-0.06, P>0.05; Norman, r=0.16, P>0.05). However, at both locations there was a positive correlation between Δ and g_{wv} (Plattsmouth, r=0.49, P<0.001; Norman, r=0.46, P<0.05). Analysis of covariance did not indicate a difference among seed sources and between locations in the response of Δ to g_{wv}.

At Plattsmouth, there was no correlation between Δ and *SLA* (r=0.09, P>0.05), but there was a weak correlation between Δ and stomatal density (r=0.30, P<0.05) (Fig. 8). At Norman, Δ was not correlated with *SLA* or stomatal density (r=-0.25, P>0.05; r=0.16, P>0.05 respectively).

The correlation between Δ and A/g_{wv} at Plattsmouth and at Norman was good (r=-0.61, P<0.001; r=0.51, P<0.001 respectively) (Fig. 9). The analysis of covariance did not indicate difference among seed sources and between locations in the correlation between Δ and A/g_{wv}.

Discussion

The ponderosa pine seed sources studied exhibited genotypic variation in Δ, water relations, *SLA* and stomatal density. We did not detect differences in A, g_{wv}, and E among seed sources, but there was a difference in A/g_{wv}. There was also a difference in gas exchange, water relations, and needle morphology between locations, but Δ was the same at both locations. Moreover, we did not find a location x seed source interaction in gas exchange. This study allowed us to test $G \times E$ interactions in Δ and needle morphology traits by the analysis of seed source x location and seed source x year interactions; however, seed source x year interaction was not significant for any of these traits, and seed source x location interaction was significant for Δ only.

The difference in A, g_{wv}, and E between locations that we observed in this study may be due mainly to effects of the equipment used for measuring gas exchange. The magnitude of difference in gas exchange rates detected between locations is very similar to that reported by Cregg and Hennessey (1995) in a comparison of the relative performance of five photosynthesis systems. They detected consistently higher gas exchange rates from the LCA-4 photosynthesis system than from the CI-301 unit. Similarly, we observed higher gas exchange rates at Plattsmouth, where the LCA-4 system was used for gas exchange measurements, than at Norman, where the CI-301 system was used. However, Cregg and Hennessey (1995) noted that the rank orders in gas exchange indicated by both photosynthesis systems is the same. Thus, the comparisons in rank orders in gas exchange within locations that we conducted in this study are valid.

The genotypic variation in Δ may be related to physiological traits and to environmental factors. In this study Δ decreased as A/g_{wv} decreased (Fig. 9), and increased as g_{wv} increased, but it was not correlated with A (Fig. 8). Although in this study the correlation of Δ with g_{wv} and A/g_{wv} was low, these values are commonly reported for forest trees and they are indicated as good correlations (Zhang and Marshall 1995 and Zhang and Marshall 1994). These low correlations may be due to the timing of the gas exchange measurements. Meinzer *et al.* (1992) indicated that phenology, leaf development, and the timing of gas exchange measurements affect the relationship between A/g_{wv} estimates made via instantaneous gas exchange and those estimated via analysis of carbon isotope discrimination. Therefore, we may speculate that variation in Δ in mature ponderosa pine trees is more related to variation in g_{wv} than to variation in A. These results are in agreement with those observed in ponderosa pine seedlings by Zhang and Marshall (1995). However, other tree species such as Douglas-fir have exhibited a strong positive correlation of Δ with both A and g_{wv} (Zhang and Marshall 1995). The positive correlation between Δ and g_{wv} is due to the greater resistance to CO_2 diffusion into the stomatal cavity as g_{wv} decreases. Therefore, the C_i/C_a ratio decreases, thus decreasing discrimination against ^{13}C (Farquhar *et al.* 1989). Although in our study Δ and g_{wv} were positively correlated at both locations, neither the difference in g_{wv} among seed sources nor the location x seed source interaction in this trait were significant. Therefore, g_{wv} did not fully explain the $G \times E$ interaction observed in Δ.

Vitousek *et al.* (1990) suggested that as *SLA* decreases, Δ will decrease as well. For example, studies with crop species such as peanut, have shown a strong positive correlation between these two traits (Nageswara Rao and Wright 1994 and Wright *et al.* 1994). This positive correlation between Δ and *SLA* may be due to the greater resistance to CO_2 diffusion from stomata to chloroplast imposed by the thicker leaves (lower *SLA*). Thus, the partial pressure of CO_2 at the fixation site in the chloroplast would be lower than the CO_2 partial pressure estimated from gas exchange (Vitousek *et al.* 1990).

Although we did not observe a significant correlation between Δ and *SLA* (Fig. 8), the years with the highest *SLA* at Plattsmouth (1993 and 1994), also showed the highest Δ. In contrast, at Norman the years with the highest *SLA* exhibited the lowest Δ (Table 3). The lack of correlation between Δ and *SLA* has been reported also in ponderosa pine seedlings (Zhang and Marshall 1995). Thus, *SLA* did not explain the variation in Δ observed in ponderosa pine.

Carbon isotope discrimination increased as stomatal density increased at Plattsmouth but not at Norman (Fig. 8). Stomatal density at Plattsmouth was the greatest in 1994, one of the years with the highest Δ. However, at this plantation, trees from seed source 767 showed lower Δ than trees from seed sources 704 and 832, but stomatal density was the same for all three seed sources. Although Dawson and Ehleringer (1993) reported that boxelder trees with high stomatal density exhibited higher Δ than trees with low stomatal density, this relationship remains unclear for ponderosa pine. We speculate that the weak correlation between Δ and stomatal density observed at Plattsmouth, and the

lack of correlation at Norman, may be due to limitations in the procedure followed to determine stomatal density.

We could expect that under dry environments, trees showing low Δ would exhibit greater growth. In fact, some studies have shown a negative correlation between Δ and tree height and diameter (Flanagan and Johnsen 1995, Zhang *et al.* 1993, and Zhang *et al.* 1994). However, we did not detect any correlation between these traits in ponderosa pine (data not shown). Thus, Δ does not explain differences in growth among the seed sources studied.

The ranking in Δ of the seed sources studied remained essentially the same through the years within each location (Fig. 7), indicating a lack of *G x E* interaction in this trait due to precipitation. These results are in agreement with those reported for black spruce by Flanagan and Johnsen (1995). They found that the ranking in Δ was the same in two years with contrasting precipitation and temperature. However, the significant location x seed source interaction in Δ that we detected in this study indicates a *G x E* interaction in this trait for ponderosa pine. In contrast to our results, most studies with crop species have shown a lack of *G x E* interaction in Δ, e.g. cowpea (Ismail and Hall 1992 and Hall *et al.* 1994), barley (Hubick and Farquhar 1989), and wheat (Condon *et al.* 1992). The *G x E* interaction in Δ that we detected also differs from the results reported by Flanagan and Johnsen (1995), who found that the ranking in Δ of black spruce trees was the same at three locations with different water availability. However, in that study the main difference among sites was water availability, and in our ponderosa pine study there are other

differences between locations related to latitude, photoperiod, soil characteristics, and nutrients that may affect a $G \times E$ interaction in Δ.

The pattern of variation in Δ between locations and among years found in this study seems to be related to the variation in the rainfall-ETp deficit during the growing season (May to October) (Figs. 2 and 7). In general, Δ was higher in trees grown at Plattsmouth than in trees grown at Norman, and correspondingly, the rainfall-ETp deficit was lower at Plattsmouth than at Norman. However, in 1992 the rainfall-ETp deficit was higher at Plattsmouth than at Norman, and Δ was higher at Norman than at Plattsmouth.

Analyzing the variation in Δ through the years within each location, we found that at Plattsmouth Δ was higher in 1993 and 1994 than in 1992, whereas the rainfall-ETp deficit was lower in 1993 and 1994 than in 1992. We found a similar relationship between Δ and the rainfall-ETp deficit at Norman. At this location, Δ was higher in 1992 and 1993 than in 1994, whereas the rainfall-ETp deficit was lower in 1992 and 1993 than in 1994 (Figs. 2 and 7). In fact, there are several reports of variation in Δ in plants of a given genotype grown under different environmental conditions such as relative humidity (Madhavan *et al.* 1991 and Winter *et al.* 1982) and vapor pressure deficit (Condon *et al.* 1992 and Zhang and Marshall 1995). Therefore, the relationship between the variation in rainfall-ETp deficit and in Δ found in this study may be related to the effects that absolute humidity deficit has on g_{wv} of mature ponderosa pine (Cregg 1993*a*).

In conclusion, we did not find genotypic variation nor $G \times E$ interaction in A, g_{wv}, and E of the ponderosa pine seed sources studied. Intrinsic water use efficiency was the

highest for the most southern seed source in the study (767), and predawn Ψ_x was also significantly high in trees from this seed source, but seed source x location interaction was significant for predawn Ψ_x. Moreover, Δ was positively correlated with g_{wv} and negatively correlated with A/g_{wv}. We speculate that the variation in Δ in ponderosa pine is more associated with g_{wv} than with A.

There was a significant genotypic variation in Δ. Trees from seed source 767 showed low Δ at both locations. In contrast, trees from seed source 856 exhibited high Δ at Plattsmouth, but low Δ at Norman; and trees from seed source 832 showed low Δ at Plattsmouth and high Δ at Norman. These results indicate a $G \times E$ interaction in Δ. Trees from seed source 704 showed high Δ at both locations.

Although there was a strong relationship between the variation in Δ and the rainfall-ETp deficit among years and between locations, the ranking in Δ of the seed sources studied was essentially the same through the years at both locations. These results indicate a lack of $G \times E$ interaction due to precipitation. Therefore, we speculate that the $G \times E$ interaction observed due to the seed source x location interaction, may be more related to latitude, photoperiod, temperature, and edaphic characteristics than to precipitation.

Acknowledgments

This research was supported by a cooperative agreement with the USDA Forest Service Rocky Mountain Forest and Range Experiment Station (Cooperative Agreement No. 28-C2-599).

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Table 1. Location and climatic data of the seed sources in the study[1].

Seed source	704	767	832	856
Locality	Reva, SD	Mayhill, NM	Recluse, WY	Arnold, NE
N latitude	45° 36'	33° 00'	44° 54'	41° 24'
W longitude	103° 12'	105° 24'	105° 36'	100° 00'
Elevation (m)	1049	1950	1186	882
Mean ann. precip. (mm)	325	437	447	503
Mean precip. (Apr-Sep.) (mm)	254	330	302	389
Mean growing season (days)	129	186	128	154

[1]Read (1980).

Table 2. Analysis of variance for net photosynthesis (A), needle conductance to water vapor (g_{wv}), transpiration (E), and intrinsic water use efficiency (A/g_{wv}) in 26-year-old trees from four *Pinus ponderosa* seed sources grown at Plattsmouth, NE and Norman, OK.

Source of variation	df	A		g_{wv}		E		A/g_{wv}	
		MS	F	MS	F	MS	F	MS	F
Between subjects									
Location (L)	1	48.99	32.17***	162405.56	365.66***	51.92	240.50***	0.1076	171.96***
Seed source (SS)	3	0.59	0.39^{ns}	612.55	1.38^{ns}	0.39	1.80^{ns}	0.0030	4.85**
L x SS	3	0.52	0.34^{ns}	170.88	0.38^{ns}	0.20	0.92^{ns}	0.0005	0.82^{ns}
Error	205	1.52		444.15		0.22		0.0006	
Within subjects									
Date (D)	2	187.40	171.18***	29871.91	127.62***	18.85	190.58***	0.0205	42.39***
D x L	2	51.15	46.73**	4070.01	17.39***	4.56	46.16***	0.0380	78.73***
D x SS	6	5.34	4.87***	865.42	3.70**	0.66	6.63***	0.0030	6.27***
D x L x SS	6	1.90	1.74^{ns}	769.72	3.29**	1.10	11.15***	0.0007	1.45^{ns}
Error	410	1.10		234.07		0.10		0.0005	

NOTE: significance levels for repeated measures (including date effect) are given as probability: ^{ns}, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Table 3. Mean specific leaf area and stomatal density in trees from four *Pinus ponderosa* seed sources grown at Plattsmouth, NE and Norman, OK.

	Location								
	Plattsmouth					Norman			
	Specific leaf area ($cm^2\ g^{-1}$)								
Seed	Year					Year			
Source	1991	1992	1993	1994	Overall	1992	1993	1994	Overall
704	58.77	58.75	63.20	71.16	62.97 yz	53.80	58.39	63.27	58.49 x
767	58.66	61.10	63.74	75.28	65.14 xy	53.93	61.00	62.84	59.78 x
832	63.10	62.61	67.39	72.35	66.36 x	55.42	60.83	54.95	57.07 x
856	54.83	57.00	58.80	74.66	61.32 z	52.67	59.21	56.92	56.27 x
Overall	58.86 c	59.84 c	63.27 b	73.32 a		53.96 b	59.86 a	59.39 a	
	Stomatal density (stomata cm^{-2})								
704	5144	5364	5321	5988	5467 y	5822	5984	6170	5992 xy
767	5777	5524	5239	5606	5526 y	6271	6107	5896	6092 x
832	5225	5350	5206	5773	5390 y	5665	5187	5719	5524 y
856	5806	5832	5905	6441	6004 x	5648	5862	6412	5974 xy
Overall	5461 b	5517 b	5431 b	5959 a		5852 a	5785 a	6049 a	

NOTE: Means within a column and within a row followed by the same letter are not significantly different at the 0.05 level. Mean differences were determined by Tukey's studentized range test.

Table 4. Analysis of variance for carbon isotope discrimination in trees from four *Pinus ponderosa* seed sources grown at Plattsmouth, NE and Norman, OK.

Source of variation	df	MS	F-value
Location (L)	1	1.2193	2.53^{ns}
Seed source (SS)	3	4.2336	8.80***
L x SS	3	3.5862	7.45***
Year (Y)	2	3.1540	6.56***
L x Y	2	5.7171	11.56***
SS x Y	6	0.1766	0.37^{ns}
L x SS x Y	6	0.1459	0.30^{ns}
Error	187	0.4811	

NOTE: significance levels are given as probability: ^{ns}, P>0.05; ***, P<0.001.

Fig. 1. Monthly mean temperature, precipitation, and potential evapotranspiration (ETp, calculated by the Thornthwaite model [Dunne and Leopold 1978]), at Plattsmouth, NE and Norman, OK (mean from 1968 to 1994).

Fig. 2. Total precipitation and potential evapotranspiration (ETp, calculated by the Thornthwaite model [Dunne and Leopold 1978]), from 1991 to 1994 at Plattsmouth, NE and Norman, OK.

Fig. 3. Seasonal variation in predawn xylem pressure potential (a) and midday xylem pressure potential (b) measured in 1994 in 26-year-old trees from four *Pinus ponderosa* seed sources grown at Plattsmouth, NE and Norman, OK.

Fig. 4. Mean predawn xylem pressure potential (a) and midday xylem pressure potential (b) measured in 1994 in 26-year-old trees from four *Pinus ponderosa* seed sources grown at Plattsmouth, NE and Norman, OK.

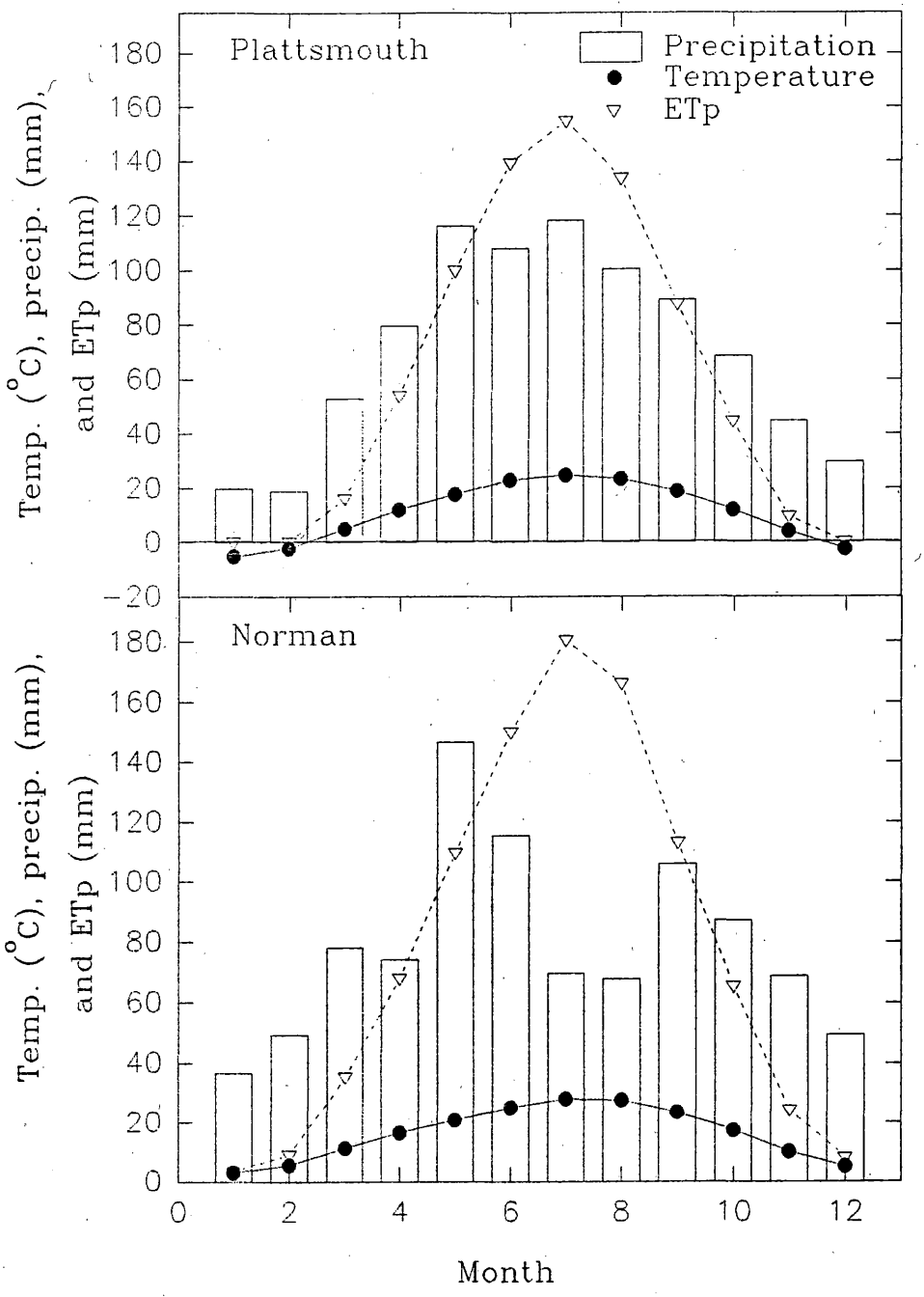
Fig. 5. Seasonal variation in net photosynthesis (a), needle conductance to water vapor (b), transpiration (c), and intrinsic water use efficiency (d) measured in 1994 in 26-year-old trees from four *Pinus ponderosa* seed sources grown at Plattsmouth, NE and Norman, OK.

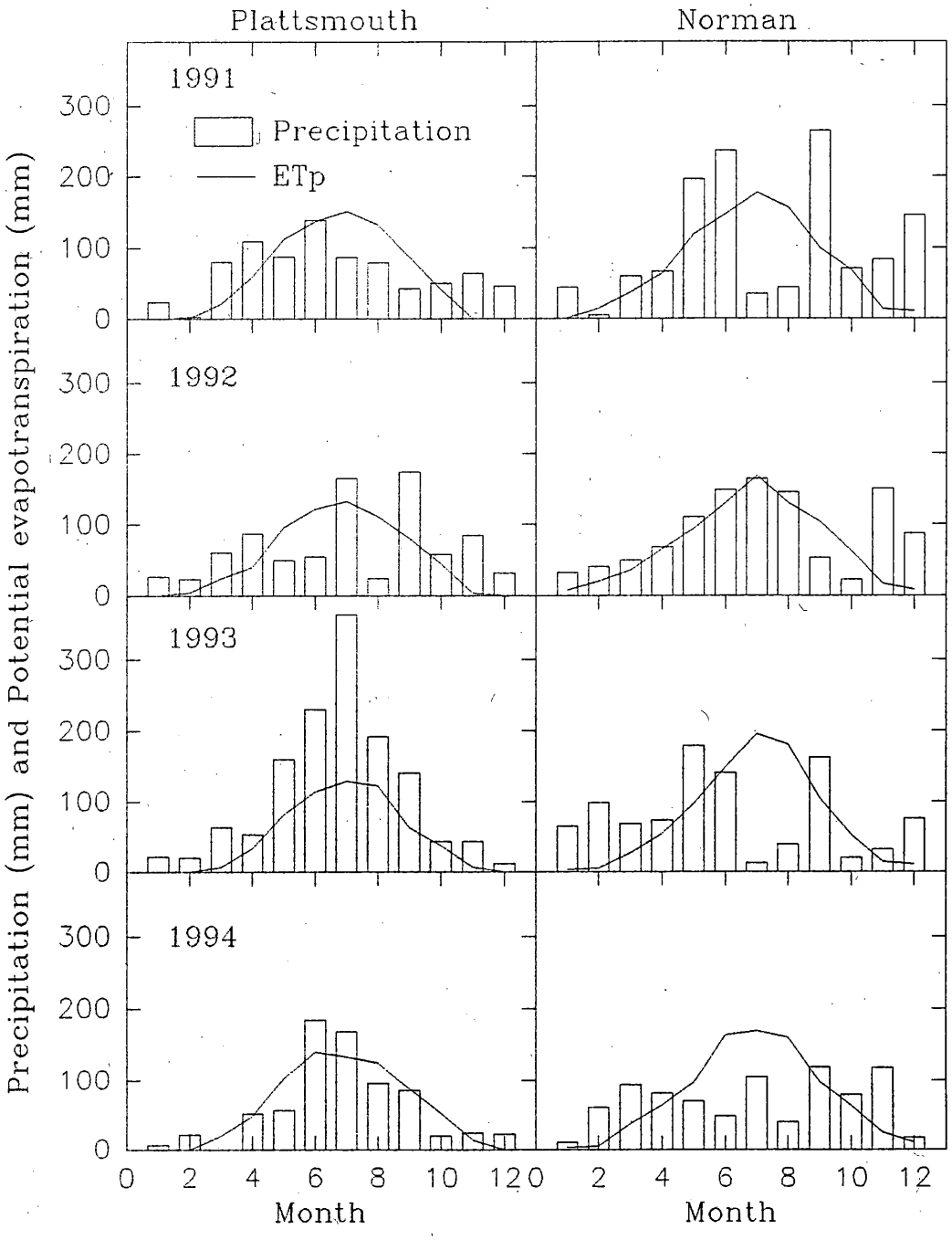
Fig. 6. Mean net photosynthesis (a), needle conductance to water vapor (b), transpiration (c), and intrinsic water use efficiency (d) measured in 1994 in 26-year-old trees from four *Pinus ponderosa* seed sources grown at Plattsmouth, NE and Norman, OK.

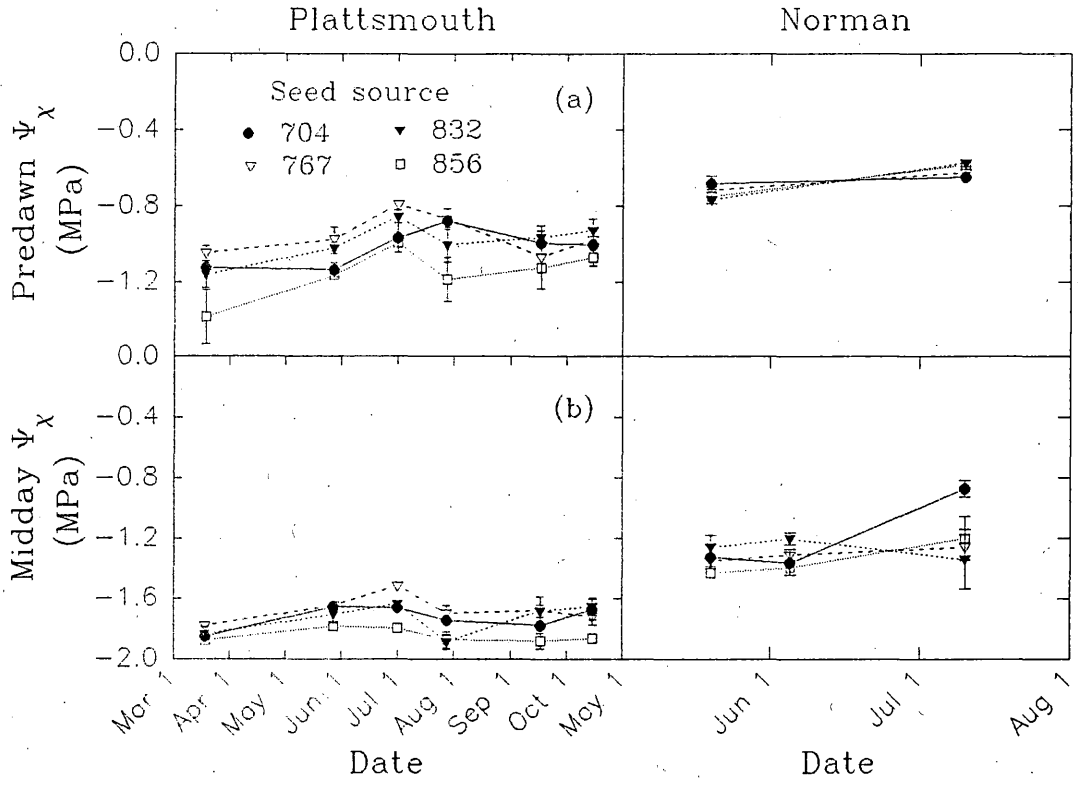
Fig. 7. Mean carbon isotope discrimination from 1991 to 1994 in needles of trees from four *Pinus ponderosa* seed sources grown at Plattsmouth, NE and Norman, OK.

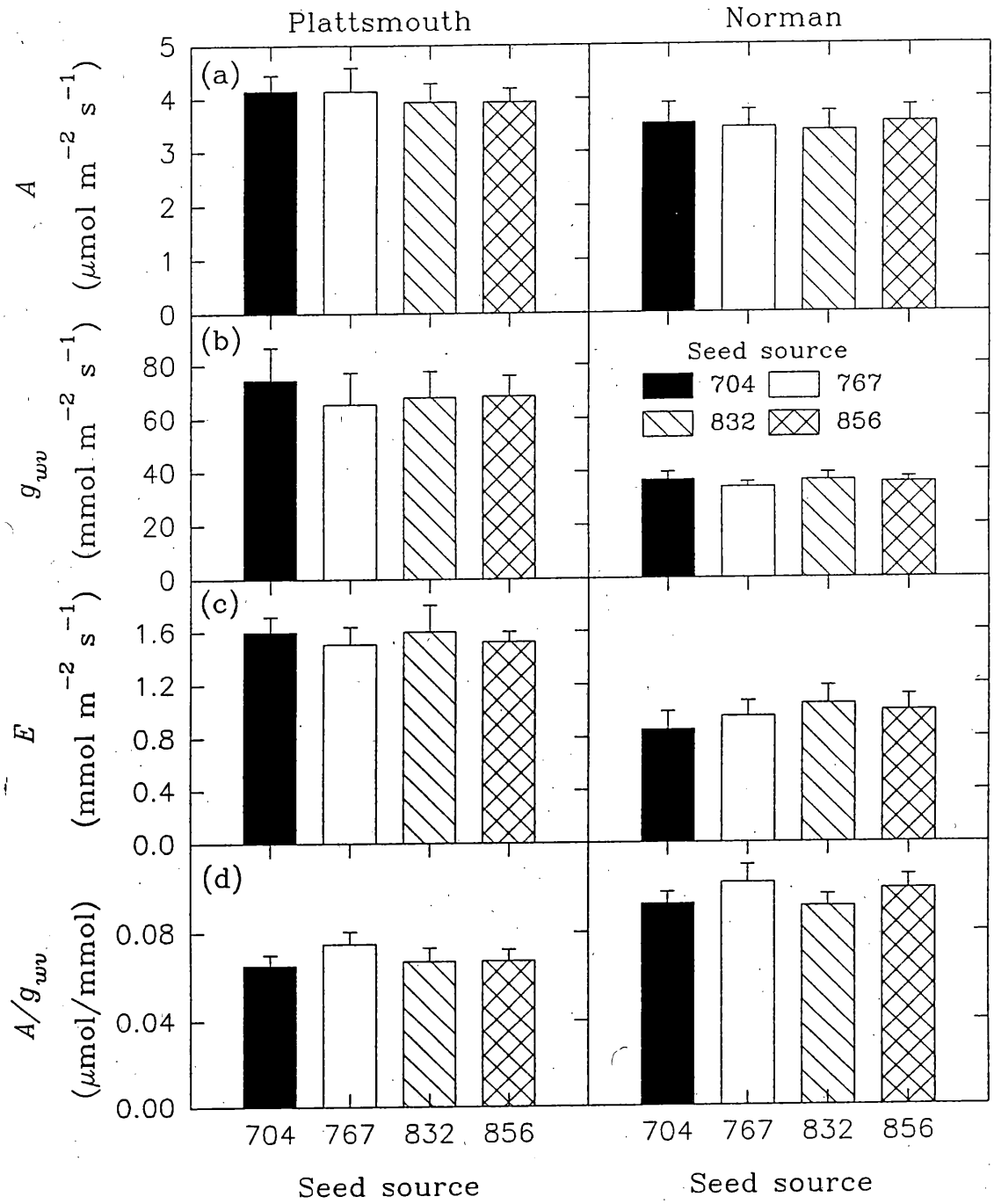
Fig. 8. Relationship between carbon isotope discrimination in needles and net photosynthesis (a, e), needle conductance to water vapor (b, f), specific leaf area (c, g), and stomatal density (d,h) in trees from four *Pinus ponderosa* seed sources grown at Plattsmouth, NE and Norman, OK. Only significant lines are shown. Regression equations are (b) g_{wv}=-62.98+7.51Δ, r^2=0.24, P<0.001; (d) stomatal density= -375.94+284.13Δ, r^2=0.09, P<0.05; (f) g_{wv}=1.63+1.87Δ, r^2=0.21, P<0.05.

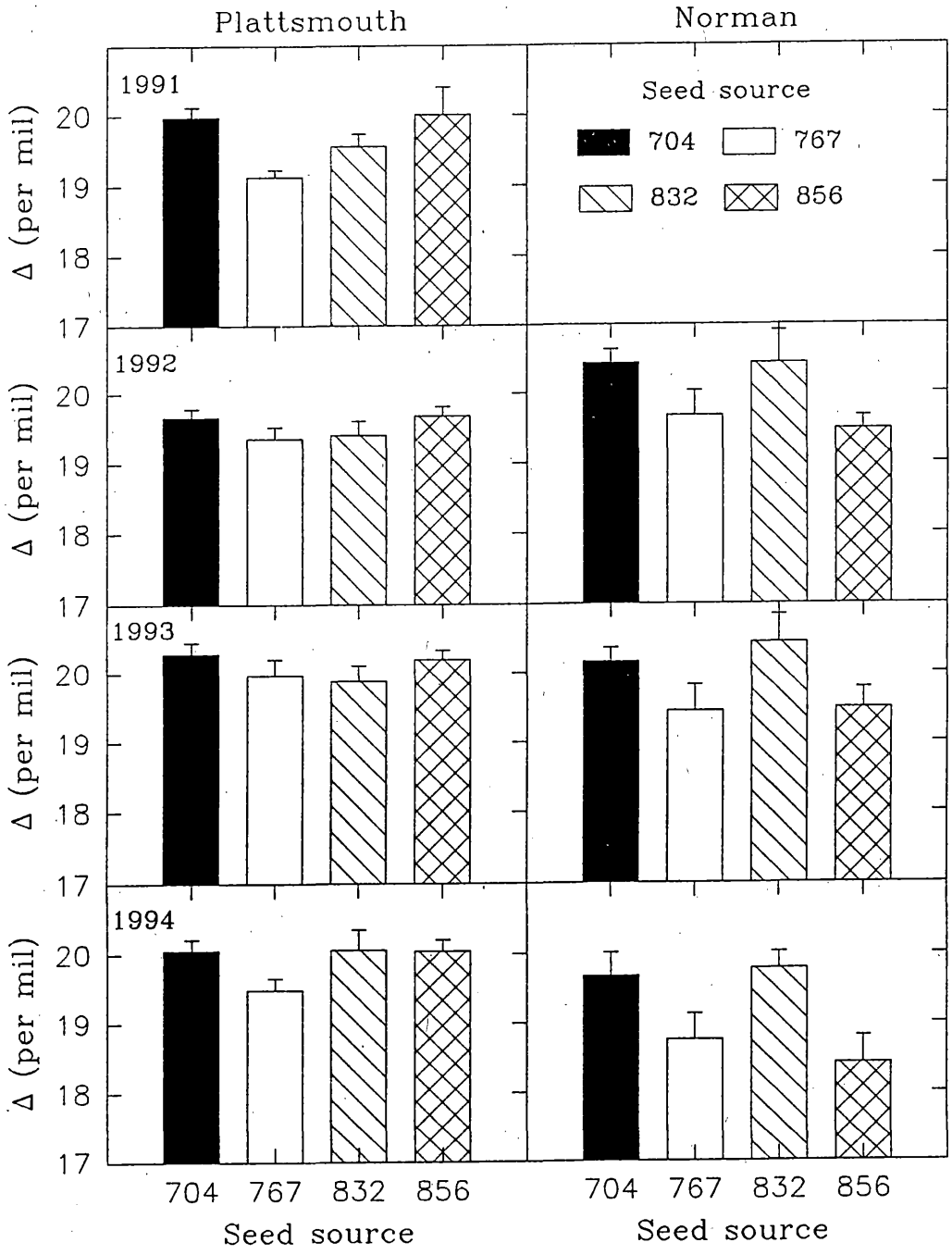
Fig. 9. Relationship between carbon isotope discrimination in needles and intrinsic water use efficiency measured in 1994 in 26-year-old trees from four *Pinus ponderosa* seed sources grown at Plattsmouth, NE and Norman, OK. Regression equations are : at Plattsmouth, Δ=23.83-66.77A/g_{wv}, r^2=0.37, P<0.001; at Norman, Δ=25.61-72.05A/g_{wv}, r^2=0.29, P<0.001.

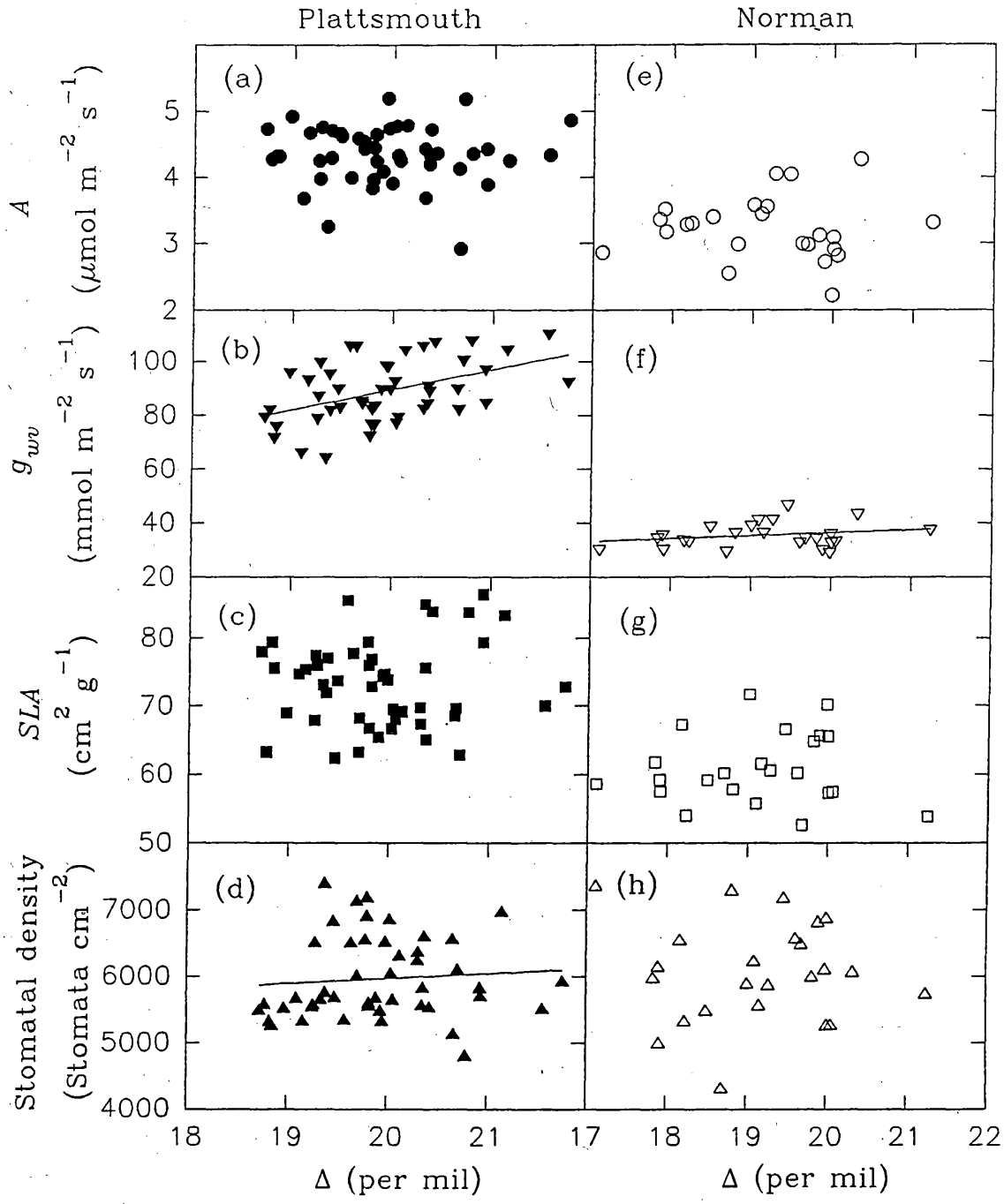

Plattsmouth
Precipitation
Temperature
ETp
Temp. (°C), precip. (mm), and ETp (mm)
180
160
140
120
100
80
60
40
20
0
-20
Norman
Temp. (°C), precip. (mm), and ETp (mm)
180
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
Month

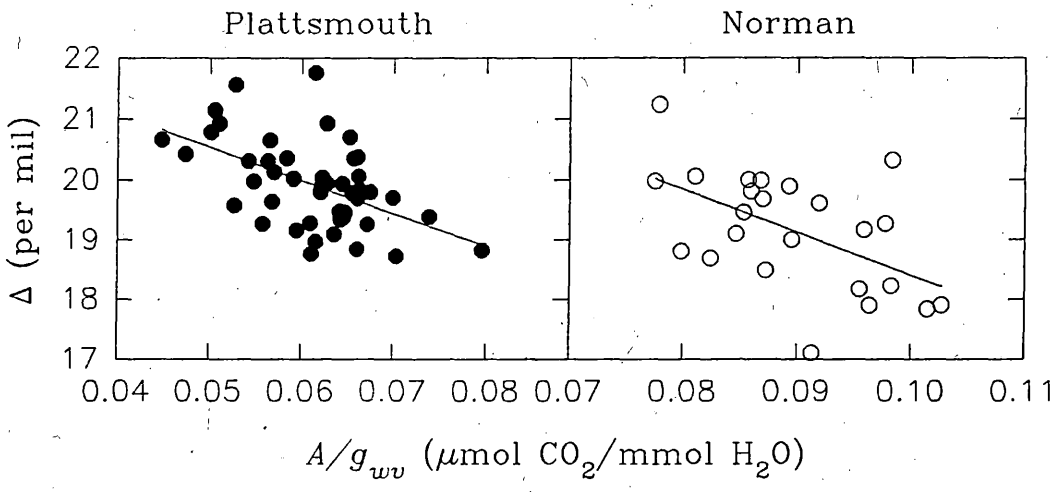

Plattsmouth
Norman
1991
1992
1993
1994
Precipitation
ETp
Precipitation (mm) and Potential evapotranspiration (mm)
0
100
200
300
0 2 4 6 8 10 12
Month


Plattsmouth
Norman
Seed source
704
767
832
856
(a)
(b)
Predawn Ψ_χ (MPa)
Midday Ψ_χ (MPa)
0.0
−0.4
−0.8
−1.2
−1.6
−2.0
Mar 1
Apr 1
May 1
Jun 1
Jul 1
Aug 1
Sep 1
Oct 1
May 1
Jun 1
Jul 1
Aug 1
Date
Date


Plattsmouth
Norman
(a)
(b)
(c)
(d)
A (μmol m⁻² s⁻¹)
g_wv (mmol m⁻² s⁻¹)
E (mmol m⁻² s⁻¹)
A/g_wv (μmol/mmol)
Seed source
704
767
832
856
704 767 832 856
Seed source
Seed source


Plattsmouth
Norman
1991
1992
1993
1994
Δ (per mil)
20
19
18
17
Seed source
704
767
832
856
704 767 832 856
Seed source


Plattsmouth
Norman
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
A (μmol m-2 s-1)
gwv (mmol m-2 s-1)
SLA (cm2 g-1)
Stomatal density (Stomata cm-2)
Δ (per mil)
Δ (per mil)


Plattsmouth
Norman
Δ (per mil)
22
21
20
19
18
17
0.04 0.05 0.06 0.07 0.08 0.07 0.08 0.09 0.10 0.11
A/g_{wv} (μmol CO_2/mmol H_2O)